a useful means for separation and characterization of serum proteins, particularly in conjunction with other methods of protein separation(17). Its use for the relative separation of various antibody activities in human sera has been described(1,2,3).

Summary. Three human sera were fractionated by gel filtration with Sephadex G-200. The distributions of 19 serum proteins or serum protein activities were studied by immunoelectrophoresis, double gel diffusion with specific antisera and specific characterization reactions. The serum proteins were separated into 3 major peaks and eluted in order essentially depending on their molecular size. The first peak contained the α_2M and β_2M macroglobulins, haptoglobin and lipoproteins. The second peak contained mostly γ-globulins and third peak consisted primarily of albumin.

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Hypophysectomy and the Lipolytic Action of Epinephrine *in vitro.** (29055)

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The prompt increase in plasma free fatty acid (FFA) concentration which follows epinephrine administration(1,2) is abolished by hypophysectomy(3,4). Pretreatment of hypophysectomized monkeys with triiodothyronine or thyrotropin restored responsiveness to epinephrine, but corticotropin and adrenal steroids were without effect in this regard(3). However, in the dog, this sequel of hypophysectomy was reversed by pretreatment with ACTH or adrenal steroids(4).

The present experiments were undertaken in an attempt further to elucidate the role of the pituitary in the lipid mobilization induced by epinephrine.

Materials and methods. Male rats weighing approximately 100 g were obtained from the Charles River Breeding Laboratories. Hypophysectomized rats were studied 2-6 weeks post-operatively. In all cases, hormone preparations were administered daily for the 5 days preceding sacrifice. The epididymal fat bodies were removed under nembutal anesthesia following a 24-hour fast. Pairs of adipose tissue

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TABLE I. Effect of Hypophysectomy and of Various Hormones on *in vitro* Lipolytic Action of Epinephrine.

Treatment of tissue donor	No. of rats	Increase in FFA release (μEq/g/hr)	p
Normal	19	3.98 ± .76*	<.001
Hypox	16	.05 ± .25	>.8
Hypox + pit. extract†	7	3.77 ± .89	<.01
Hypox + growth hormone			
4.0 mg/rat/day†	8	3.30 ± .68	<.01
1.0 " " "	12	1.64 ± .44	<.01
.05 " " "	6	.19 ± .34	>.6
Hypox + corticotropin			
4 U/rat/day (aqueous)†	8	.34 ± .33	>.3
10 " " " (gelatin)	8	.64 ± .30	>.05
Hypox + thyrotropin†			
2 U/rat/day	8	1.65 ± .29	<.001
Hypox + pituitrin			
5 U/rat/day (gelatin)	6	.81 ± .33	>.05
Hypox + triiodothyronine			
.5 μg/rat/day	8	1.74 ± .25*	<.001
1.0 " " "	8	2.45 ± .49	<.01
5.0 " " "	15	4.26 ± .52	<.001
Hypox + cortisol acetate			
1 mg/rat/day I.P.	14	.85 ± .22	<.01
1 " " " S.C.	13	3.38 ± .48	<.01
5 " " " I.P.	8	5.13 ± 1.36	<.01
5 " " " S.C.	8	3.12 ± .77	<.01
Hypox + corticosterone			
1 mg/rat/day S.C.	8	.80 ± .17	<.01
5 " " " S.C.	8	6.36 ± .72‡	<.001

* Mean ± S.E.
† In 2 injections/day.
‡ Significantly greater ($p < .05$) than normal.

segments from each animal were incubated for 4 hours at 37°C in Krebs-Ringer phosphate buffer containing 5% bovine serum albumin. Epinephrine (as the hydrochloride) in a final concentration of 0.05 μg/ml was added to the medium bathing one member of each pair and an equal volume of water was added to the other. Analyses for FFA were performed in duplicate by the method of Dole (1) and 1 ml aliquots of medium. The effect of epinephrine is expressed as the difference in FFA released/hour/g wet weight between epinephrine-treated and control tissues. Tests for statistical significance were performed according to the method for paired analyses(5).

Results and discussion. Epinephrine, at the concentration chosen for this study, caused a significant increase in FFA production by adipose tissue obtained from normal rats, but was without effect on adipose tissue from hypophysectomized rats (Table I). Two daily subcutaneous injections of rat pituitary homogenate in saline, equivalent to 1 pituitary per injection, for 5 days prior to sacrifice fully restored the responsiveness of adipose tissue from hypophysectomized rats to epinephrine (Table I). Bovine growth hormone (2 mg/rat),‡ when administered twice daily, brought about a full restoration of the lipolytic response of adipose tissue to epinephrine. This dose approximates the growth hormone content of the rat pituitary as estimated from the data of Solomon and Greep(6). At lower doses, however, growth hormone, while causing substantial weight gain, failed to reestablish the normal response of adipose tissue to epinephrine (Table II).

‡ NIH BGH-2, a gift of Endocrinology Study Section, Nat. Inst. of Health.

TABLE II. Effect of Various Pituitary Preparations on Body and Organ Weights in Hypophysectomized Rats.

Treatment	No. of rats	Adrenals (pr) (mg/100 g)	Thyroid (mg/100 g)	Wt gain (g/rat/day)
Normal	9	27.61 ± 1.87*	7.77 ± .63	4.9 ± .6
Hypox—untreated	9	9.73 ± .35‡	6.34 ± .21	.1 ± .4‡‖
Hypox + pit extract§	7	9.76 ± .51‡	9.70 ± .80†	5.6 ± .5†
Hypox + growth hormone				
4 mg/rat/day§	8	—	—	6.6 ± .5†
1 " " "	12	—	—	3.1 ± .2†‡
.05 " " "	6	—	—	1.6 ± .3‡
Hypox + corticotropin				
4 U rat/day (aqueous)§	8	13.48 ± .96†‡	—	—
10 " " " (gelatin)	8	21.52 ± .90†	—	—
Hypox + thyrotropin				
2 U/rat/day§	8	11.98 ± .82‡	9.86 ± .63†	−.4 ± .4‡

* Mean ± S.E.
† Significantly different ($p < 0.05$) from hypox untreated.
‡ Significantly different ($p < 0.05$) from normal.
§ Two injections/rat/day.
‖ 5 animals.

Four units of corticotropin§ administered twice daily in aqueous solution, a dose exceeding the estimated corticotropin content of the whole pituitary extract(7), did not alter the responsiveness of adipose tissue from hypophysectomized rats to epinephrine (Table I). Similarly, 10 U/rat/day administered subcutaneously in 10% gelatin was without effect although both treatments brought about significant adrenal enlargement. It should be noted that no adrenal enlargement was seen when the whole pituitary extract was administered (Table II).

Thyrotropin‖ administered twice daily induced a small but significant increase in response to epinephrine (Table I). This dose caused thyroidal enlargement of a magnitude comparable to that observed when the whole pituitary extract was administered (Table II).

A posterior pituitary preparation, Pituitrin¶, when administered at a daily dose of 5 U in 10% gelatin did not significantly augment the lipolytic effect of epinephrine (Table I).

Triiodothyronine, when administered in doses of 1 and 5 μg/day, fully restored sensitivity to epinephrine (Table I). Pitt-Rivers (8) reported that 1 μg of triiodothyronine per day was required to restore a normal growth rate in thyroidectomized rats, suggesting that this dose approximates the normal rate of thyroid hormone secretion.

Adrenal steroids also increased the sensitivity of adipose tissue from hypophysectomized rats to epinephrine (Table I). While 1 mg of cortisol acetate per day induced only a slight restoration of the lipolytic activity of epinephrine when administered intraperitoneally, subcutaneous injection of the same dose returned it to normal levels. When the daily dose of cortisol was increased to 5 mg, a normal response to epinephrine was obtained whether the intraperitoneal or subcutaneous route was used. One mg of corticosterone per day injected subcutaneously had a small but statistically significant effect on the response of adipose tissue to epinephrine, while 5 mg/day resulted in significantly greater than normal lipolysis. These doses of adrenal steroids caused a loss in body weight exceeding 1 g per day and are considered to be in excess of physiologic replacement.

Although the effectiveness of whole pituitary extract in restoring the responsiveness of hypophysioprivic adipose tissue to the lipolytic action of epinephrine *in vitro* may be

§ Wilson corticotropin lot #104529, 130 units/mg.
‖ Armour & Co., lot #R-216-174-1, 1.1 units/mg.
¶ Parke-Davis & Co.

accounted for by its growth hormone content, it appears clear that a number of other endocrine factors are operative in this regard. The functional interactions between the thyroid as well as the adrenal cortical hormones and the catechol amines have been extensively documented(9,10). With reference to lipolysis, adrenalectomy reduces and adrenal cortical hormone treatment restores the rise in plasma FFA in response to epinephrine administration in the dog(4) and the rat (Goodman and Knobil, unpublished). A reduced lipolytic response to epinephrine *in vitro* and *in vivo* has been observed in hypothyroid subjects(11,12,13,14), results consonant with our earlier finding that TSH and triiodothyronine administration to hypophysectomized monkeys restored the fatty acid mobilizing action of epinephrine(3). In these animals, however, cortisol replacement was ineffective in contrast to the observation of Shafrir and Steinberg(4) in the hypophysectomized dog.

The available information does not permit a quantitative assignation of physiological importance to the multiple endocrine factors which appear to permit optimal responses by adipose tissue to the activation of the sympathoadrenal medullary system.

Summary. The response of normal adipose tissue to epinephrine (0.05 μg/ml incubation medium) is completely abolished by hypophysectomy of the donor rats. Treatment of hypophysectomized rats with crude, whole rat pituitary extract restored the lipolytic action of epinephrine to normal. Posterior pituitary extract and corticotropin replacement was without effect. Thyrotropin injections produced a partial, and growth hormone treatment a full restoration of the lipolytic action of epinephrine. Treatment of hypophysectomized rats with cortisol or corticosterone reestablished the sensitivity of their adipose tissue to epinephrine, but only when relatively large doses of these hormones were given. Physiological doses of triiodothyronine (1 μg/rat/day), however, restored the normal response to epinephrine. It is concluded that several endocrine factors can influence the lipolytic action of epinephrine but that their physiological importance cannot yet be quantitated.

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